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Original Article

5-O-Methylgenistein of *Phaleria macrocarpa* (Scheff.) Boerl. revealed as a potential therapeutic compound for endometriosis

[5-O-Metilgenisteína de *Phaleria macrocarpa* (Scheff.) Boerl. revelado como un posible compuesto terapéutico para la endometriosis]

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Abstract

Context: Endometriosis is a chronic estrogen-dependent inflammatory disorder associated with pelvic pain and infertility. Flavonoids from *Phaleria macrocarpa* have been reported to exhibit anti-inflammatory activity; however, their molecular mechanisms relevant to endometriosis remain insufficiently characterized.

Aims: To predict biological activities of selected *P. macrocarpa* flavonoids and to explore potential protein–ligand interactions with endometriosis-relevant targets using molecular docking.

Methods: Six flavonoids (eriodictyol, glycitin, 5-O-methylgenistein, catechin 7-O-β-D-xyloside, 8-prenylnaringenin, and naringenin) were assessed using the PASS web server to estimate biological activity probabilities (Pa/Pi). The compound with the highest predicted anti-inflammatory probability (Pa > 0.7) was selected for docking against cyclooxygenase-2 (COX-2), estrogen receptor (ER), AKT, aryl hydrocarbon receptor (AHR), and caspase-3 using Molegro Virtual Docker. Docking protocol validation was performed by redocking co-crystallized ligands and evaluating RMSDs.

Results: PASS prediction indicated that all compounds exhibited potential anti-inflammatory activity (Pa > Pi), with 5-O-methylgenistein showing the highest confidence prediction (Pa = 0.838). Docking analysis suggested that 5-O-methylgenistein can occupy the binding sites of COX-2, ER, AKT, and AHR, showing comparable interaction patterns and overlapping residues with those of reference ligands, as indicated by software-derived scores. Redocking validation confirmed the acceptable reproduction of the pose (RMSD ≤ 2.0 Å).

Conclusions: 5-O-methylgenistein was identified as a promising candidate for further investigation. The predicted interactions support hypotheses related to inflammation- and hormone-associated pathways in endometriosis; however, these findings are based on computational models and require experimental validation to confirm biological activity and therapeutic relevance.

Keywords: AKT; cyclooxygenase-2; endometriosis; molecular docking; *Phaleria macrocarpa*; phytoestrogens.

Resumen

Contexto: La endometriosis es un trastorno inflamatorio crónico dependiente de estrógenos asociado con dolor pélvico e infertilidad. Se ha informado que los flavonoides de *Phaleria macrocarpa* exhiben actividad antiinflamatoria; sin embargo, sus mecanismos moleculares relevantes para la endometriosis aún no están suficientemente caracterizados.

Objetivos: Predecir las actividades biológicas de flavonoides seleccionados de *P. macrocarpa* y explorar posibles interacciones proteína-ligando relevantes para la endometriosis mediante acoplamiento molecular.

Métodos: Se evaluaron seis flavonoides (eriodictiol, glicitina, 5-O-metilgenisteína, catequina 7-O-β-D-xilósido, 8-prenilnaringenina y naringenina) mediante el servidor web PASS para estimar las probabilidades de actividad biológica (Pa/Pi). El compuesto con la mayor probabilidad antiinflamatoria prevista (Pa > 0,7) se seleccionó para acoplarse a la ciclooxigenasa-2 (COX-2), el receptor de estrógeno (ER), AKT, el receptor de aril hidrocarburo (AHR) y la caspasa-3 mediante Molegro Virtual Docker. La validación del protocolo de acoplamiento se realizó mediante el reacoplamiento de ligandos cocristalizados y la evaluación del RMSD.

Resultados: La predicción PASS indicó que todos los compuestos exhibieron actividad antiinflamatoria potencial (Pa > Pi), y la 5-O-metilgenisteína mostró la predicción de confianza más alta (Pa = 0,838). El análisis de acoplamiento sugirió que la 5-O-metilgenisteína puede ocupar los sitios de unión de COX-2, ER, AKT y AHR, mostrando patrones de interacción comparables y residuos superpuestos con los de los ligandos de referencia, como lo indican las puntuaciones del software. La validación del reacoplamiento confirmó una reproducción aceptable de la pose (RMSD ≤ 2,0 Å).

Conclusiones: La 5-O-metilgenisteína fue identificada como una candidata prometedora para investigación adicional. Las interacciones previstas respaldan hipótesis relacionadas con las vías asociadas a la inflamación y con las hormonas en la endometriosis; sin embargo, estos hallazgos se basan en modelos computacionales y requieren validación experimental para confirmar su actividad biológica y su relevancia terapéutica.

Palabras Clave: acoplamiento molecular; AKT; ciclooxigenasa-2; endometriosis; fitoestrógenos; *Phaleria macrocarpa*.

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Abbreviations: ABC: ATP-binding cassette; ADMET: Absorption, distribution, metabolism, excretion, and toxicity; AHR: Aryl hydrocarbon receptor; AKT: Protein kinase B; ALA: Alanine; ARG: Arginine; ASN: Asparagine; BBB: Blood-brain barrier; BCRP: Breast cancer-resistant protein; COX-2: Cyclooxygenase-2; CYP450: Cytochrome P450; CYS: Cysteine; ERE: Estrogen response element; ER: Estrogen receptor; FDA: Food and Drug Administration; GLU: Glutamic acid; GLY: Glycine; hERG: Human Ether-à-go-go-Related Gene; HIS: Histidine; LCSC: Liver cancer stem cells; LD50: Lethal dose 50; LEU: Leucine; LYS: Lysine; MET: Methionine; MVD: Molegro Virtual Docker; NSAID: Non-steroidal anti-inflammatory; OCT2: Organic cation transporter 2; PGE2: Prostaglandin E2; PASS: Prediction of Activity Spectra for Substances; PDB: Protein data bank; P-gp: P-glycoprotein; PHE: Phenylalanine; RMSD: Root mean square deviation; ROS: Reactive oxygen species; SMILES: Simplified molecular input line entry system; TRP: Tryptophan; TYR: Tyrosine; VD: Volume distribution.

INTRODUCTION

Endometriosis is a chronic gynecological disease characterized by the abnormal growth of tissue resembling the endometrium — including glands and stroma — outside the uterine cavity (Gazvani & Templeton, 2002; Morotti et al., 2017; Nisenblat et al., 2016; Sahraei et al., 2022). The condition affects approximately 15% of women of reproductive age and is primarily manifested by two debilitating clinical outcomes: chronic pelvic pain, experienced by up to 70–80% of patients, and infertility, which affects up to 50% of those diagnosed (Gazvani & Templeton, 2002; Husain et al., 2015; Morotti et al., 2017). The etiopathogenesis of endometriosis is multifactorial, involving congenital, autoimmune, epigenetic, and environmental contributors (Malvezzi et al., 2020; Parazzini et al., 2017; Vallvé-Juanico et al., 2019).

A central driver of endometriosis pathophysiology is estrogen-dependent hormonal dysregulation. Abnormally elevated estrogen signaling disrupts normal menstrual cycling and promotes dysmenorrhea, predisposing affected women to retrograde menstruation — a condition characterized by the reflux of endometrial fragments through the fallopian tubes into the peritoneal cavity (Agarwal & Subramanian, 2010; Chantalat et al., 2020; Mahnke et al., 2000; Rocha et al., 2012; Sutrisno et al., 2019). In women with endometriosis, retrograde menstruation is typically more voluminous and more frequent than in unaffected individuals, resulting in greater accumulation of ectopic endometrial tissue within the peritoneal environment (Mahnke et al., 2000; Rocha et al., 2012; Sutrisno et al., 2019). The implantation of ectopic endometrial tissue triggers a pronounced, dysregulated immune response. Under physiological conditions, innate immune cells — including macrophages, neutrophils, dendritic cells, and natural killer cells — play essential roles in clearing menstrual debris and maintaining peritoneal homeostasis (Capobianco & Rovere-Querini, 2013). However, in women with endometriosis, these innate immune responses are fundamentally dysregulated, resulting in chronic, unresolved peritoneal inflammation that drives the pain and tissue damage characteristic of the disease (Capobianco & Rovere-Querini, 2013; Smolarz et al., 2021). This inflammatory milieu is further compounded by oxidative stress: endometrial lesions stimulate excessive reactive oxygen species (ROS) production, upregulate prostaglandin E2 (PGE2), and induce local acidosis. Elevated PGE2 directly stimulates pelvic sensory nerve fibers, perpetuating the perception of chronic visceral pain and establishing a self-reinforcing cycle of inflammation, oxidative stress, and nociception (Capobianco & Rovere-Querini, 2013; Maddern et al., 2020; Moga et al., 2019).

Within this pathophysiological network, cyclooxygenase-2 (COX-2), the estrogen receptor (ESR), and AKT occupy particularly central positions as molecular mediators of endometriosis progression. COX-2 is markedly overexpressed in ectopic endometrial lesions in response to estrogenic stimulation, inflammatory cytokines, and oxidative stress. COX-2-derived PGE2 reinforces a paracrine loop that further stimulates local estrogen biosynthesis through aromatase upregulation, simultaneously amplifying lesion growth and perpetuating the nociceptive and inflammatory environment (Lai et al., 2019). The ESR is equally indispensable in endometriosis pathogenesis, driving the proliferation and survival of ectopic endometrial cells, stimulating vascularization of lesions through angiogenesis, and sustaining the upregulation of pro-inflammatory mediators, including COX-2. Dysregulation of estrogen signaling extends beyond reproductive consequences — its systemic effects on cholesterol mobilization, immune modulation, and tissue development underscore the broad pathological impact of estrogenic dysfunction in endometriosis (Chantalat et al., 2020). Protein kinase B (AKT), a central node of the PI3K/AKT/mTOR signaling axis, is aberrantly activated in endometriotic tissue in response to estrogenic and inflammatory stimuli, promoting cell survival, resistance to apoptosis, and angiogenesis — collectively enabling the progressive growth and invasiveness of ectopic lesions (An et al., 2024). The convergence of COX-2, ESR, and AKT in sustaining endometriosis pathophysiology identifies these proteins as high-priority molecular targets for therapeutic intervention.

Accumulating evidence from *in vitro* and *in vivo* studies supports the therapeutic potential of medicinal plant-derived compounds in targeting these

pathological mechanisms. A broad range of plant species has been investigated, including *Andrographis paniculata*, ginger, *Centella asiatica*, and species from the families *Asteraceae*, *Adoxaceae*, *Apiaceae*, *Boraginaceae*, *Hypericaceae*, *Lamiaceae*, *Malvaceae*, *Paeoniaceae*, *Rosaceae*, *Rubiaceae*, *Verbenaceae*, and *Zingiberaceae* (Bina et al., 2019; Ilhan et al., 2019; Kong et al., 2014; Maddern et al., 2020; Meresman et al., 2021; Smolarz et al., 2021; Tsai et al., 2017; Zheng et al., 2020). The proposed mechanisms of these plants include the suppression of inflammatory signaling, downregulation of pro-inflammatory cytokines, and inhibition of cyclooxygenase expression (Bina et al., 2019; Kong et al., 2014; Meresman et al., 2021). Among plant-derived constituents, flavonoids have emerged as particularly promising therapeutic candidates due to their multi-target pharmacological profiles. Epigallocatechin gallate, curcumin, and puerarin have been shown to reduce VEGF expression in endothelial cells and ectopic endometrium, thereby attenuating the angiogenesis essential for lesion establishment (Ilhan et al., 2019; Kong et al., 2014; Tsai et al., 2017). Resveratrol has been reported to enhance antioxidant capacity and inhibit the release of pro-inflammatory cytokines, while genistein — a structurally related isoflavone — has been shown to decrease the expression of estrogen receptors, VEGF, and HIF-1α in peritoneal tissue, implicating isoflavones in the modulation of both estrogenic and angiogenic signaling (Meresman et al., 2021). Conventional hormonal therapies, such as progestins, which act by inducing amenorrhea and preventing repeated menstrual reflux, are associated with significant side effects and limited long-term tolerability (Reis et al., 2020), underscoring the clinical need for novel, plant-derived alternatives with favorable safety profiles.

Phaleria macrocarpa (Scheff.) Boerl. (family *Thymelaeaceae*) is a tropical medicinal plant whose compounds have demonstrated antioxidant, anti-aging, anti-inflammatory, anti-angiogenic, and antimicrobial activities (Alara & Olalere, 2016; Amir et al., 2010; Andrean et al., 2014; Hassan et al., 2020; Lay et al., 2014; Maharani et al., 2021; Tandrasasmita et al., 2015). Previous phytochemical investigation identified six flavonoid compounds in *P. macrocarpa*: Eriodictyol, glycitin, 5-O-methylgenistein, catechin 7-O-beta-D-xyloside, 8-prenylnaringenin, and naringenin (Maharani et al., 2021). *In vivo* evidence has further suggested that flavonoids from *P. macrocarpa* fruit hold potential as therapeutic agents specifically for endometriosis. Among these, 5-O-methylgenistein presents the most compelling profile for mechanistic investigation. As a methylated derivative of genistein, it retains the isoflavone scaffold associated with estrogen receptor binding, anti-inflammatory, and antioxidant activities, while the methoxy substitution at the 5-position may confer enhanced metabolic stability and receptor binding affinity. Its predicted multi-target activity profile — encompassing modulation of COX-2-mediated inflammatory signaling, engagement of the estrogen receptor binding domain, and interference with AKT-dependent survival signaling — maps directly onto the key pathophysiological mechanisms of endometriosis described above. Nevertheless, the precise molecular mechanisms through which 5-O-methylgenistein exerts these effects remain to be elucidated. Therefore, the present study aimed to systematically characterize the therapeutic mechanisms of flavonoids from *P. macrocarpa*, with a particular focus on 5-O-methylgenistein, against endometriosis using molecular docking, to provide a mechanistic foundation for its further development as a phytotherapeutic agent.

MATERIAL AND METHODS

Data retrieval

Protein targets associated with endometriosis were selected based on their established roles in key pathological processes, including hormone dependence, inflammation, cell survival, and environmental response. The estrogen receptor (PDB ID: 1A52) was included due to its central role in estrogen-dependent lesion growth and maintenance (Chantalat et al., 2020). Cyclooxygenase-2 (COX-2, PDB ID: 5F19) was selected as a major mediator of prostaglandin-driven inflammation and pain in endometriosis (Lai et al., 2019). AKT (PDB ID: 1H10) was chosen based on its involvement in cell proliferation, survival signaling, and resistance to apoptosis in endometriotic tissues (Kim et al., 2014). In addition, the aryl hydrocarbon receptor (AHR, PDB ID: 5NJ8) was included due to its roles in endocrine disruption, immune modulation, and environmental toxicant-mediated lesion development, which have been increasingly linked to the pathophysiology of endometriosis (Kim, 2024). Caspase-3 (PDB ID: 1CP3), a key effector of apoptotic pathways, was also analyzed to explore potential interactions with apoptosis-related machinery; however, its functional modulation cannot be inferred solely from docking and requires further experimental validation.

All protein structures were retrieved from the Protein Data Bank (PDB). Bioactive compounds from *Phaleria macrocarpa*, including eriodictyol (CID: 440735), glycitin (CID: 187808), 5-O-Methylgenistein (CID: 5748551), catechin 7-O-β-D-xyloside (CID: 73533), 8-prenylnaringenin (CID: 480764), and naringenin (CID: 932), as well as the positive control, an FDA-approved dienogest (CID: 68861), were obtained from the PubChem database.

Bioactive prediction

Bioactivity properties of *P. macrocarpa* compounds were predicted using the PASS Online web server (Prediction of Activity Spectra for Substances; GeneXplain GmbH, http://www.way2drug.com/passonline/) (Sari et al., 2022). The canonical SMILES of each compound, retrieved from the PubChem database, were used as input for prediction. The analysis focused on anti-inflammatory and antineoplastic (anticancer) activities. PASS provides outputs in the form of the probability of activity (Pa) and the probability of inactivity (Pi) for each predicted biological activity. In this study, predicted activities with Pa > Pi were considered potentially active, while compounds with Pa > 0.7 were prioritized as having higher confidence for the selected biological activities. These selected compounds were subsequently used for molecular docking analysis.

Molecular docking

Protein preparation

Five protein targets associated with endometriosis were retrieved from the Protein Data Bank and prepared prior to docking. For each structure, the biologically relevant chain containing the active site was selected. Co-crystallized ligands were used to define the binding pocket and subsequently removed for docking simulations. All water molecules were removed, except those involved in ligand-binding interactions, while structurally important cofactors were retained when present. Protein structures were examined for missing residues, and only those with intact binding sites were included. Hydrogen atoms were added, and protonation states were assigned at physiological pH (~7.4) to ensure appropriate electrostatic representation.

Ligand preparation

All ligands were obtained from the PubChem database and prepared prior to docking. Ligand structures were assigned appropriate protonation states and tautomeric forms at physiological pH (~7.4). Each ligand was subjected to energy minimization using the built-in optimization tools in Molegro Virtual Docker. Rotatable bonds were defined to allow conformational flexibility, enabling efficient exploration of binding conformations during docking simulations.

Binding site identification

The active sites of each protein were identified based on the co-crystallized ligand positions supported by the built-in cavity detection algorithm in Molegro Virtual Docker, with the molecular surface defined by van der Waals interactions (Bitencourt-Ferreira & de Azevedo, 2019). The detected cavities corresponding to the native ligand-binding regions were selected as docking sites to ensure biologically relevant ligand placement.

Docking validation

To validate the reliability of the docking protocol, a redocking procedure was performed for each protein target using its corresponding co-crystallized (native) ligand. Native ligands were re-docked into the same binding site using identical grid definitions and docking parameters. The predicted binding poses were compared with the experimental crystallographic conformations by calculating the root-mean-square deviation (RMSD). The docking protocol was considered valid when the reproduced pose showed RMSD ≤ 2.0 Å, indicating good agreement with the native binding mode (Fig. 1) (Arsul et al., 2025). Native ligands used for validation included arachidonic acid for COX-2, estradiol for estrogen receptor, inositol for AKT, and N-acetyl-L-alpha-aspartyl-L-valyl-N-[(1S)-1-(carboxymethyl)-3-fluoro-2-oxopropyl]-L-alaninamide for caspase-3.

Docking protocol

Ligand–protein docking simulations were performed using Molegro Virtual Docker (MVD) version 5.0, selected for its integrated cavity detection algorithm and the MolDock optimization engine, which has been reported to provide reliable pose prediction and reproducibility in structure-based docking studies (Bitencourt-Ferreira & de Azevedo, 2019). The docking grid for each target was defined based on the detected binding cavities, as summarized in Table 1. The MolDock optimizer was applied with a grid resolution of 0.30 Å. Docking parameters were set as follows: a maximum of 5 poses per ligand, an RMSD threshold of 1.0 Å for pose clustering, 300 iterations for the simplex evolution algorithm, an energy threshold of 100, and a minimum of 10. All docking simulations were conducted using identical parameter settings across all ligands and protein targets to ensure consistency and comparability. The resulting complexes were evaluated using MolDock score and Re-rank score, which are software-defined scoring functions employed to rank ligand binding and pose stability. These values were used for relative comparison only and were not interpreted as absolute binding free energies.

Absorption, distribution, metabolism, excretion, and toxicity (ADMET)

The ADMET properties of the six selected compounds from *P. macrocarpa* were predicted using the pkCSM web server (http://biosig.unimelb.edu.au/pkcsm/) based on their Simplified Molecular Input Line Entry System

(SMILES) retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The analysis included absorption (water solubility, Caco-2 permeability, human intestinal absorption, and skin permeability), distribution (volume of distribution, blood–brain barrier permeability, and central nervous system permeability), metabolism (cytochrome P450 enzyme inhibition and substrate prediction), excretion (total clearance and renal OCT2 substrate), and toxicity (Ames mutagenicity, hepatotoxicity, skin sensitization, and maximum tolerated dose). The predicted values, summarized in Tables 2 and 3, were compared across compounds to assess their drug-likeness and pharmacokinetic suitability, with particular attention to oral absorption, CYP-mediated metabolism, and potential safety liabilities such as hERG inhibition or mutagenicity. All ADMET outcomes represent in silico predictions and are considered preliminary, requiring further experimental validation to confirm pharmacokinetic behavior and toxicological profiles in biological systems.

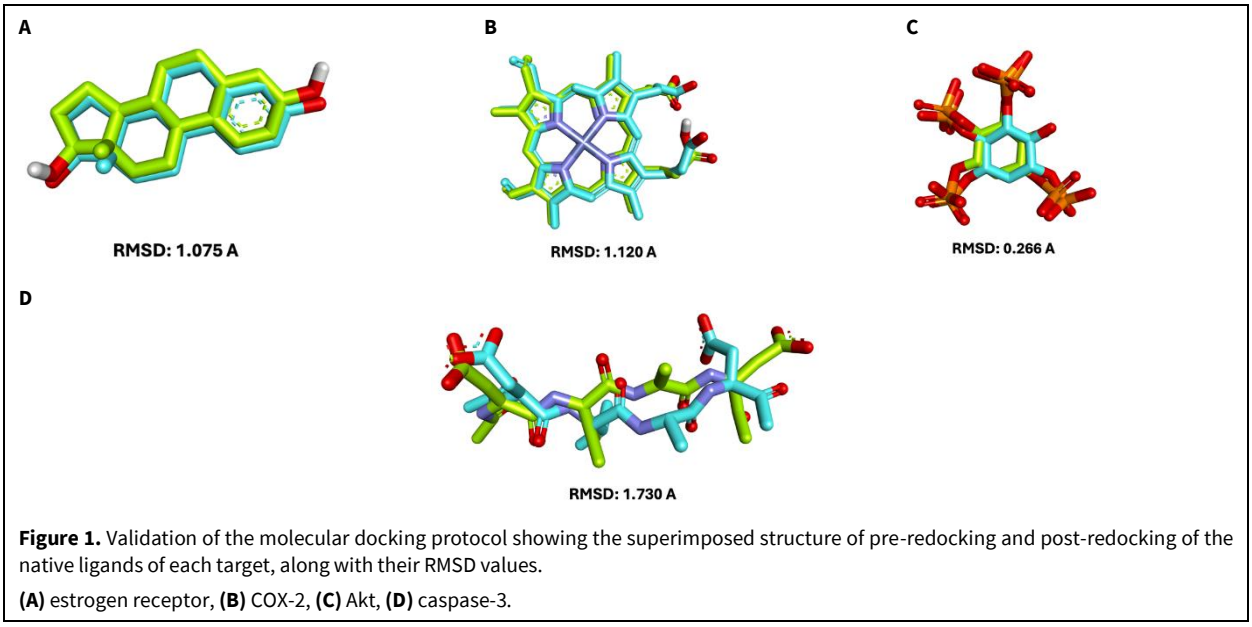


Figure 1. Validation of the molecular docking protocol showing the superimposed structure of pre-redocking and post-redocking of the native ligands of each target, along with their RMSD values.

(A) estrogen receptor, **(B)** COX-2, **(C)** Akt, **(D)** caspase-3.

Table 1. Protein, docking grid, and radius.

Protein	Grid (Å)	Radius
COX-2	X = 24.69; Y= 57.42; Z= 0.52	10
Caspase-3	X = 24.69; Y= 57.42; Z= 50.10	12
Estrogen receptor	X = 105.98; Y= 13.93; Z= 96.68A	12
AKT	X = 21.70; Y= 15.82; Z= 11.71	15
AHR	X = 28.45; Y= 13.15; Z= 207.50	15

Table 2. Molecular properties of compounds *Phaleria macrocarpa*.

Compound	Molecular weight (Dalton)	LogP	H bond acceptor	H bond donor	Surface area
Dienogest (control)	311.425	3.83718	3	1	137.619
Eriodictyol	288.255	2.2155	6	4	119.029
Glycitin	446.408	0.3529	10	5	180.997
5-O-Methylgenistein	284.267	2.8798	5	2	119.203
Catechin 7-O-β-D-xyloside	422.386	-0.3417	10	7	170.297
8-Prenylnaringenin	340.375	4.0186	5	3	145.370
Naringenin	272.256	2.5099	5	3	114.235

Table 3. Predicted ADMET properties of six bioactive compounds from *Phaleria macrocarpa* obtained using pkCSM.

Category	Property/model	Dienogest (control)	Eriodictyol	Glycitin	5-O-Methyl-genistein	Catechin 7-O-beta-D-xyloside	8-Prenyl-naringenin	Naringenin	Unit
Absorption	Water solubility	-4.585	-3.253	-2.902	-3.307	-3.029	-3.567	-3.224	log mol/L
	Caco-2 permeability	1.372	-0.094	0.303	1.14	0.222	1.304	1.029	log Papp (10^{-6} cm/s)
	Intestinal absorption (human)	95.586	74.687	51.094	95.62	43.198	91	91.31	% Absorbed
	Skin permeability	-3.328	-2.735	-2.735	-2.74	-2.735	-2.739	-2.742	log Kp
	P-gp substrate	No	Yes	Yes	Yes	Yes	Yes	Yes	Categorical
	P-gp I inhibitor	Yes	No	No	No	No	No	No	Categorical
	P-gp II inhibitor	No	No	No	No	No	No	No	Categorical
Distribution	VDss (human)	0.361	0.377	-0.108	0	1.652	0.272	-0.015	log L/kg
	Fraction unbound (human)	0.154	0.106	0.067	0.038	0.258	0.017	0.064	Fu
	BBB permeability	-0.039	-0.827	-1.464	-0.305	-1.398	-0.925	-0.578	log BB
	CNS permeability	-1.778	-3.142	-3.974	-2.218	-4.019	-2.13	-2.215	log PS
Metabolism	CYP2D6 substrate	No	No	No	No	No	No	No	Categorical
	CYP3A4 substrate	Yes	No	No	Yes	No	Yes	No	Categorical
	CYP1A2 inhibitor	No	No	No	Yes	No	Yes	Yes	Categorical
	CYP2C19 inhibitor	Yes	No	No	Yes	No	Yes	No	Categorical
	CYP2C9 inhibitor	No	No	No	Yes	No	No	No	Categorical
	CYP2D6 inhibitor	No	No	No	No	No	No	No	Categorical
	CYP3A4 inhibitor	No	No	No	Yes	No	No	No	Categorical
Excretion	Total clearance	1.069	-0.013	0.147	0.301	-0.051	0.119	0.06	log mL/min/kg
	Renal OCT2 substrate	Yes	No	No	No	No	No	No	Categorical
Toxicity	AMES toxicity	No	No	No	No	No	No	No	Categorical
	Max. tolerated dose (human)	-0.656	0.014	0.475	0.199	0.47	-0.246	-0.176	log mg/kg/day
	hERG I inhibitor	No	No	No	No	No	No	No	Categorical
	hERG II inhibitor	Yes	No	Yes	Yes	No	Yes	No	Categorical
	Oral Rat Acute Toxicity (LD_{50})	2.033	2.03	2.495	2.248	2.58	1.957	1.791	mol/kg
	Oral Rat Chronic Toxicity (LOAEL)	1.662	2.475	3.787	1.761	3.489	1.744	1.944	log mg/kg_bw/day
	Hepatotoxicity	No	No	No	No	No	No	No	Categorical
	Skin sensitisation	No	No	No	No	No	No	No	Categorical
	T. pyriformis toxicity	1.087	0.332	0.285	0.396	0.285	0.536	0.369	log µg/L
	Minnow toxicity	0.714	2.972	3.331	0.633	6.027	1.624	2.136	log mM

Data analysis

All *in silico* data were analyzed using a comparative approach to evaluate the potential of compounds for the treatment of endometriosis. Docking validation was performed by redocking native ligands into their respective binding sites, with accuracy assessed by RMSD (≤ 2.0 Å). Docking results were evaluated using MolDock score and Re-rank score as software-defined metrics for ranking ligand–protein interactions. These scores were not interpreted as absolute binding free energy. Ligand–protein interactions were analyzed based on key residues and types of interactions (e.g., hydrogen bonding and hydrophobic contacts). Drug-likeness was assessed using Lipinski and Veber criteria, while ADMET properties were predicted using pkCSM based on SMILES input. All results were presented in tables (docking scores, ADMET, drug-likeness) and figures (binding poses and interaction profiles), with dienogest used as a reference for comparison. Dienogest was docked to all protein targets using the same protocol, and its docking scores and interactions were used as pharmacological reference for comparison with the six flavonoid compounds from *P. macrocarpa*. Findings are reported as computational predictions requiring further experimental validation.

RESULTS AND DISCUSSION

Bioactivity properties of *P. macrocarpa* bioactive compounds

The bioactivity profiles of six flavonoid compounds from *Phaleria macrocarpa* were predicted using the PASS web server. Each compound is represented as follows: (1) eriodictyol, (2) glycitin, (3) 5-O-methylgenistein, (4) (+)-catechin 7-O-β-D-xyloside, (5) 8-prenylnaringenin, and (6) (±)-naringenin. The heat map visualization shows predicted activity probabilities (Pa), with higher Pa values indicating a greater likelihood of biological activity. In PASS analysis, Pa and Pi are used to estimate the likelihood that a compound exhibits a given biological effect. Predictions are considered meaningful when Pa > Pi, with confidence levels generally interpreted as follows: Pa > 0.7 indicates high confidence, whereas 0.5 < Pa < 0.7 suggests moderate or potential activity requiring further validation. Accordingly, the results of this study are interpreted based on these thresholds. As shown in Fig. 2, all six flavonoids demonstrated predicted anti-inflammatory and antioxidant activities with Pa values exceeding Pi, although most compounds fell within the moderate confidence range (0.5–0.7). Notably, compound 3 (5-O-methylgenistein) exhibited a Pa value of 0.838 for one of the predicted activities, exceeding the 0.7 threshold and indicating a higher-confidence prediction relative to the other compounds in the dataset.

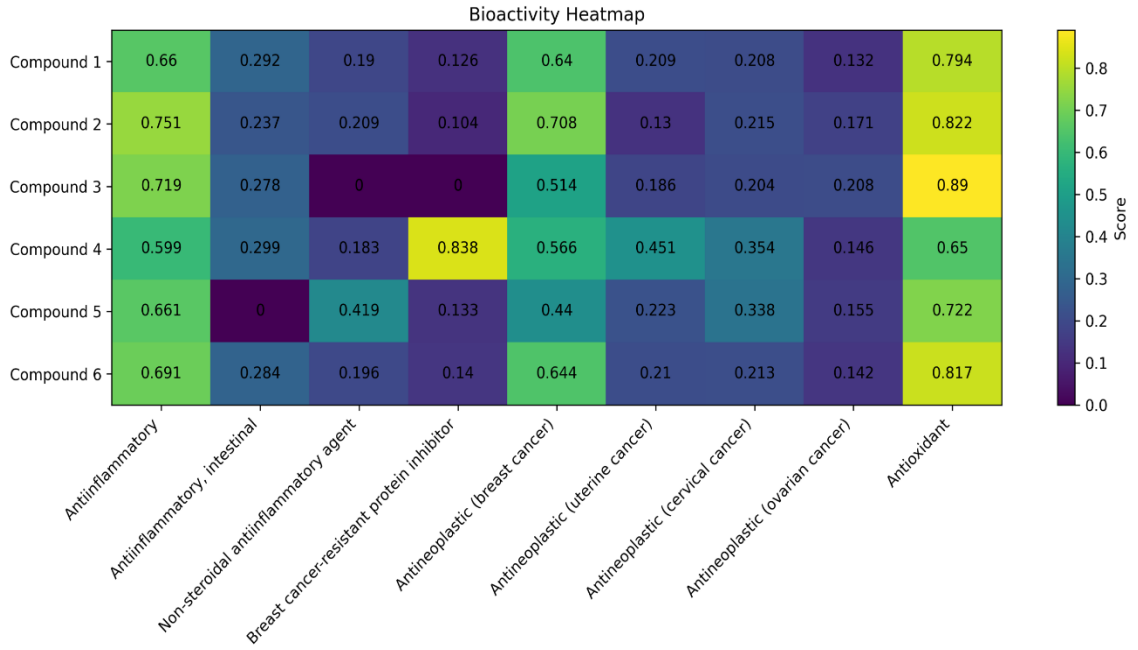


Figure 2. Anti-inflammatory, anticancer, anti-neoplastic, and antioxidant prediction activities of *Phaleria macrocarpa* flavonoid compounds.

Compounds: **1.** Eriodictyol, **2.** Glycitin, **3.** 5-O-Methylgenistein, **4.** (+)-Catechin 7-O-beta-D-xyloside (-), **5.** 8-Prenylnaringenin, **6.** (±)-Naringenin.

In addition, several compounds showed predicted activity related to non-steroidal anti-inflammatory drug (NSAID)-like mechanisms within the moderate probability range. These findings are consistent with the established role of flavonoids as modulators of inflammatory pathways. Chronic inflammation is recognized as a key contributor to various pathological conditions, including endometriosis, where dysregulated inflammatory signaling supports lesion survival and pain development (Greten & Grivennikov, 2019). Therefore, the predicted anti-inflammatory activity of these compounds may be relevant to endometriosis-related mechanisms as evidenced by a meta-analysis of observational studies on NSAIDs that revealed a lower risk of cancer among NSAID users (Bosetti et al., 2020; Hou et al., 2021).

In addition to anti-inflammatory activity, antioxidant activity has also been linked to a lower risk of cancer. Antioxidants can eliminate ROS, thereby preventing cellular damage (Singh et al., 2018). At elevated levels, ROS can reach a toxic threshold and impair the cellular antioxidant system. These ROS induce significant changes in cellular function and metabolism via signaling pathways, promoting genomic instability and immunosuppression — conditions under which carcinogenesis may occur. However, this process can be mitigated by the provision of exogenous antioxidants, such as those derived from bioactive compounds (Ferdous & Yusof, 2021; Srinivas et al., 2019). In this study, bioactivity predictions for *P. macrocarpa* flavonoids indicated that these compounds exhibit antioxidant activity. Notably, (+)-catechin 7-O-beta-D-xyloside appeared white in the heat map despite having the highest antioxidant score. The antioxidant properties of these flavonoids are presumed to reduce the risk of cancer through the inhibition of ROS production (Kopustinskiene et al., 2020).

Interestingly, 5-O-methylgenistein was predicted to be a high-activity inhibitor of breast cancer resistance protein (BCRP). BCRP is a major ATP-binding cassette (ABC) transporter protein that is frequently targeted in cancer therapy. It is overexpressed in cancer cells and functions as an efflux protein, capable of limiting oral bioavailability and blood–brain barrier transport. The presence of BCRP inhibitors is known to improve drug absorption; in BCRP knockout mice, intestinal BCRP inhibition was associated with increased bioavailability of BCRP substrates (Mao & Unadkat, 2015). A study by Yin et al. (2021) demonstrated the possibility of eliminating liver cancer stem cells (LCSCs) through inhibition of MDR1 or ABCG2 — also ATP-binding cassette transporters — which led to doxorubicin accumulation and increased LCSC apoptosis.

Considering the bioactivity predictions for each flavonoid, 5-O-methylgenistein demonstrated the most distinctive performance across anti-inflammatory and antineoplastic activities potentially relevant to endometriosis, with predicted BCRP-inhibitory activity and antioxidant activity. Therefore, 5-O-methylgenistein was selected for virtual screening and docked with proteins associated with anti-inflammatory, antioxidant, BCRP inhibition, and antineoplastic mechanisms.

5-O-Methylgenistein of *P. macrocarpa* in pathways relevant to endometriosis

To validate the pkCSM bioactivity predictions, molecular docking was performed to identify the interactions of 5-O-methylgenistein with each target protein. Five proteins were selected to represent distinct anticancer mechanisms. COX-2 is an inflammatory mediator protein whose expression has been detected in various cancer cell types (Gandhi et al., 2017). COX-2 is involved in apoptosis, proliferation, survival, metastasis, and angiogenesis. Overexpression of COX-2 leads to inflammation, apoptotic failure, uncontrolled cell proliferation, and metastasis, ultimately driving cancer progression (Negi et al., 2019; Satoh et al., 2013; Szweda et al., 2019). Consequently, COX-2 inhibitors are considered important in preventing cancer cell growth.

The results in Table 4 and Fig. 3 indicate that 5-O-methylgenistein was predicted to bind to COX-2 with a less favorable Re-rank score compared to the control, dienogest. Despite the difference in docking scores, 5-O-methylgenistein was predicted to occupy a similar binding region and share several interacting residues with the native ligand, including ARG44, LEU152, and LYS468. These overlapping interactions suggest a comparable binding orientation within the active site. However, it should be emphasized that these findings reflect predicted binding interactions based on docking simulations and do not indicate binding affinity in thermodynamic units or confirm inhibitory activity.

Beyond inflammation, abnormal cell survival and impaired apoptosis are key features of endometriotic lesion persistence, allowing ectopic cells to evade normal cell-death mechanisms. Loss of apoptotic control allows cancer cells to survive and accumulate mutations, which can further stimulate angiogenesis and deregulate cell proliferation. Cysteinyl aspartate-specific proteinase-3 (caspase-3) is a key executioner protease in the apoptosis pathway, responsible for proteolytic cleavage of cellular substrates leading to chromatin condensation and DNA fragmentation (Jiang et al., 2020; Ponder & Boise, 2019). Dysregulation of apoptotic processes has been implicated in the persistence of endometriotic lesions, where impaired cell death contributes to abnormal tissue survival.

Table 4. Interaction of 5-O-methylgenistein with endometriosis-related targets.

Complex interaction	Docking score (kJ/mol)	Hydrogen bond	Hydrophobic, electrostatic
5-O-Methylgenistein - COX2	-278.25	GLN42 (3.2), ARG44 (3.2), GLU465 (1.84), ASP125 (1.75), LYS468 (2.9)	CYS41 (2.9), TYR130 (5.7), LYS468 (3.9), LEU152 (5.0), ARG44 (4.6)
Dienogest - COX2	-292.88	THR212 (4.45)	HIS207 (6.71), HIS388 (5.29)
5-O-Methylgenistein - Caspase-3	-258	ARG64 (2.8), CYS163 (3.0; 2.7), ARG207 (3.3; 2.8), SER120 (1.7), GLN161 (2.7), GLN161 (3.6)	ARG207 (3.4), TRP206 (4.5), HIS121 (5.4; 3.9), PHE256 (5.6), CYS163 (3.8), ALA162 (5.3), CYS163 (5.0; 5.3), ARG207 (4.9; 4.6)
Dienogest -Caspase-3	-326.35	ARG64 (5.53), GLN161 (3.82), ARG207 (4.67)	PHE256 (6.71), TRP206 (6.77)
5-O-Methylgenistein - ESR	-183.342	ARG394 (2.3), HIS524 (2.68), LEU387 (2.6), GLY521 (2.04), LEU346 (1.94),	MET388 (5.45), MET421 (5.89), PHE404 (5.73; 5.18; 4.6), LEU346 (4.15), LEU349 (3.76), ALA350 (5.3), LEU384 (5.4; 5.2), LEU387 (5.0; 4.17), LEU391 (5.47), LEU391 (4.6), LEU525 (5.3; 2.6)
Dienogest - ESR	-301.13	GLY521 (3.54)	MET388 (6.39), LEU391 (6.53), PHE404 (5.43), GLU353 (5.38)
5-O-Methylgenistein - AKT	-203.5	LYS14 (2.6; 2.3), ASN53 (2.9), ASN54 (1.8),	ARG86 (3.8), LYS14 (4.4), GLU17 (2.6), GLN79 (2.6)
Dienogest - AKT	-242.67	ARG48 (4.43), LYS30 (5.47)	-
5-O-Methylgenistein - AHR	-233.75	GLN171 (2.2)	PHE170 (3.7), LEU265 (2.8), ALA133 (4.4), TYR159 (4,9), VAL129 (4.4), LEU265 (5.0), ALA264 (5.4)
Dienogest - AHR	-359.82	SER68 (2.72), LYS63 (2.49)	-

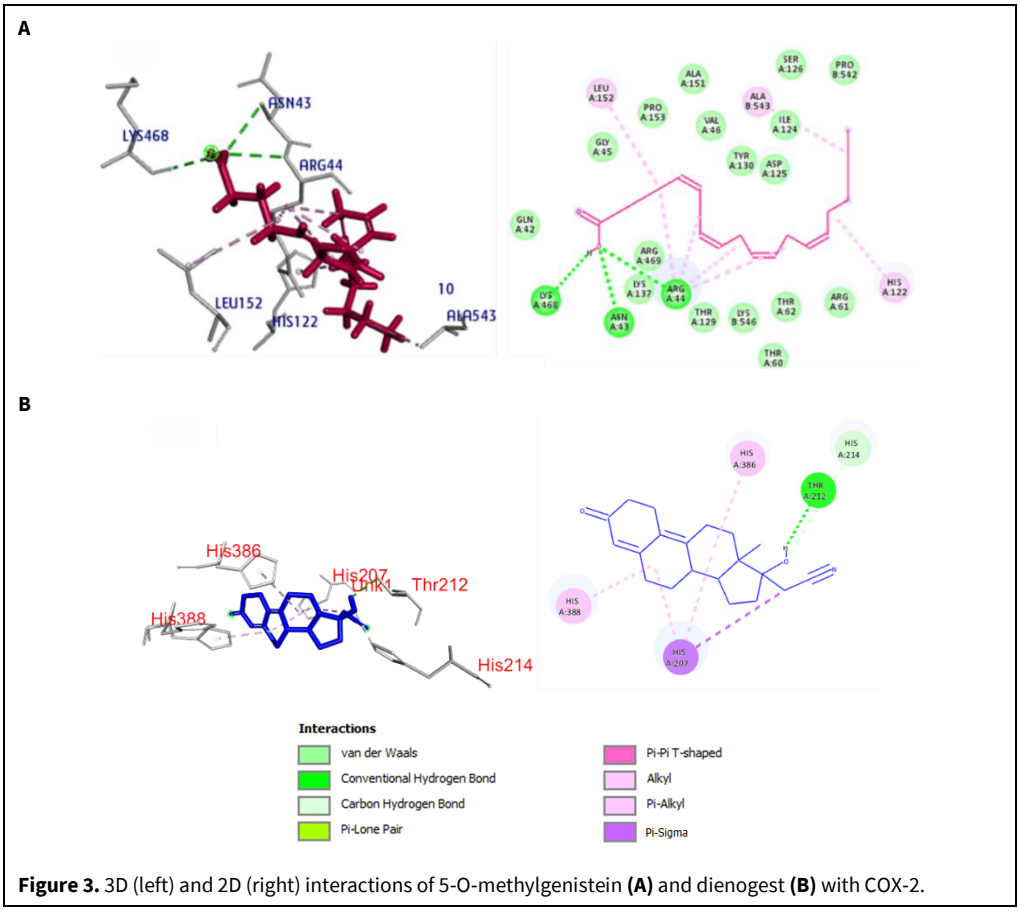


Figure 3. 3D (left) and 2D (right) interactions of 5-O-methylgenistein **(A)** and dienogest **(B)** with COX-2.

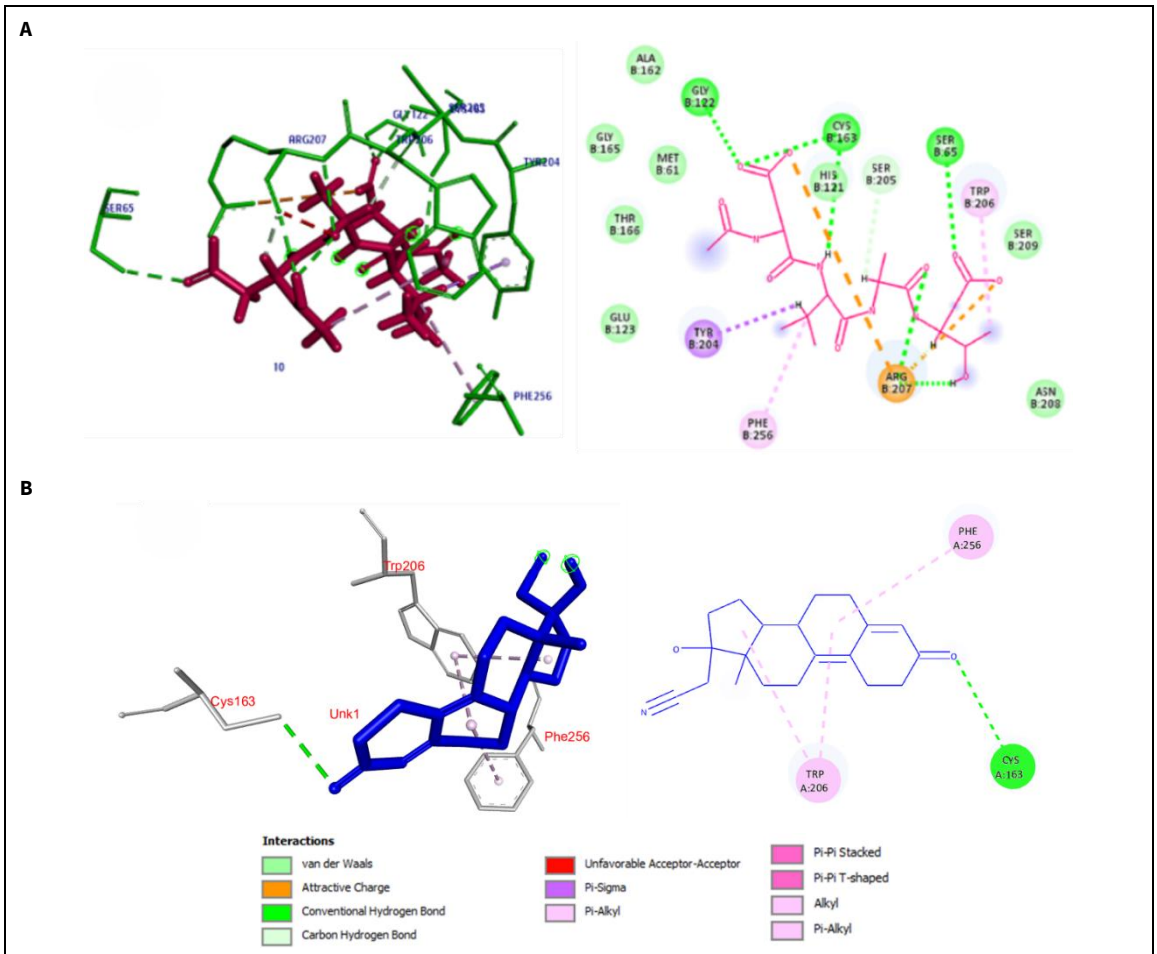


Figure 4. 3D (left) and 2D (right) interactions of 5-O-methylgenistein **(A)** and dienogest **(B)** with caspase-3.

In this study, molecular docking suggested that 5-O-methylgenistein may bind within the caspase-3 binding pocket, with several shared interacting residues (CYS163, ARG207, TRP206, and PHE256) (Table 4, Fig. 4). Since flavonoids are known to promote apoptosis (Ablimit et al., 2022; Bosetti et al., 2020; Fuentes & Silveyra, 2019), 5-O-methylgenistein is expected to act as a caspase-3 stimulator rather than an inhibitor. Although the binding site overlap indicates a similar docking region, this finding does not provide information on the functional consequences of binding. In particular, molecular docking cannot distinguish whether the compound acts as an activator, inhibitor, or has no functional effect on caspase-3 activity. Therefore, the interaction between 5-O-methylgenistein and caspase-3 should be interpreted only as a predicted binding event, and no conclusion regarding apoptosis induction can be drawn without further biochemical and cellular validation of whether this interaction results in activation or inhibition.

The interaction of 5-O-methylgenistein with the estrogen receptor (ER) showed a less favorable The rerank score was compared with the positive control, dienogest, which serves as a pharmacological reference with established binding affinity. Importantly, 5-O-methylgenistein was predicted to occupy a similar binding site within the ER ligand-binding domain. The interacting residues identified in the 5-O-methylgenistein–ER complex included ARG394, HIS524, LEU387, GLY521, LEU346, MET388, MET421, PHE404, ALA350, LEU384, LEU391, and LEU525 (Table 4, Fig. 5).

The overlap in binding site and interacting residues suggests a comparable binding orientation within the receptor pocket. However, this structural similarity does not imply functional equivalence, receptor activation, or modulation of estrogen levels. Estrogen signaling plays a critical role in regulating reproductive physiology and inflammation, and its dysregulation has been associated with pathological conditions, including hormone-dependent disorders (Fuentes & Silveyra, 2019; Jiang et al., 2020; Nephew et al., 2000; Satoh et al., 2013). Therefore, the interaction between 5-O-methylgenistein and ER should be interpreted only as a predicted binding event, and any potential impact on estrogen signaling remains hypothetical and requires further experimental validation.

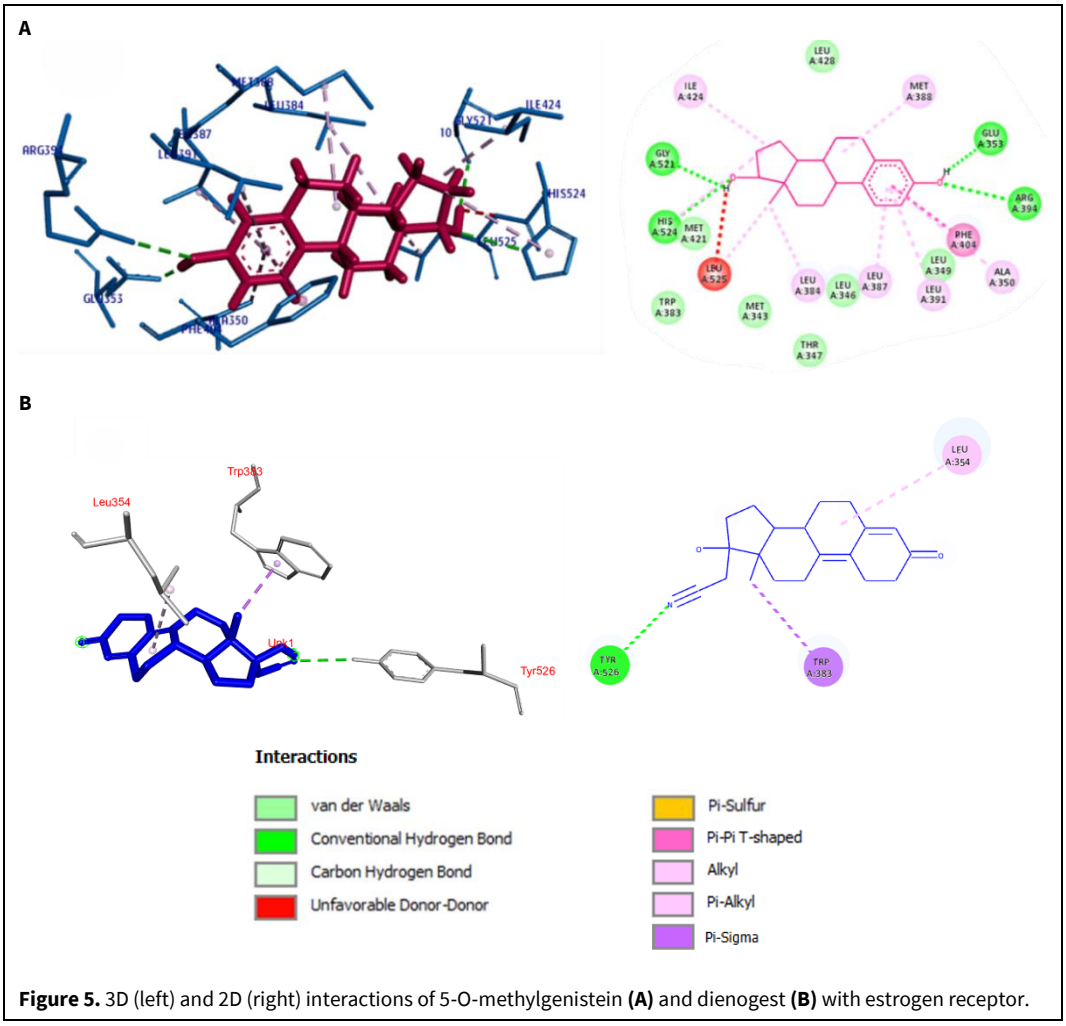


Figure 5. 3D (left) and 2D (right) interactions of 5-O-methylgenistein **(A)** and dienogest **(B)** with estrogen receptor.

The relevance of 5-O-methylgenistein to endometriosis-related survival signaling is further supported by the predicted interaction with AKT shown in Fig. 6. 5-O-Methylgenistein interacted with AKT through the residues LYS14, ASN53, ASN54, ARG86, GLU17, and GLN79, while the control drug, dienogest, bound through GLN79, GLU17, ILE19, TYR18, and ARG23. These findings suggest that 5-O-methylgenistein may bind at the same site as the natural AKT ligand, although the binding energy scores of the two interactions differed considerably (Table 4).

AKT is a well-characterized serine/threonine kinase that plays a central role in regulating cellular processes such as metabolism, growth, survival, and proliferation (Ablimit et al., 2022; Vara et al., 2004; Wang et al., 2021). Its activation is primarily mediated by the phosphoinositide 3-kinase (PI3K) pathway, in which phosphatidylinositol (3,4,5)-trisphosphate (PIP3) facilitates the recruitment and phosphorylation of AKT at the plasma membrane. Dysregulation of the PI3K/AKT signaling pathway has been widely associated with abnormal cell survival and proliferation, including in the persistence of endometriosis-related lesions. In this study, molecular docking suggested that 5-O-methylgenistein may occupy the AKT binding pocket and share several interacting residues with the reference ligand. However, these findings represent only a predicted binding interaction and do not imply direct modulation of AKT activity. Therefore, any potential effect of 5-O-methylgenistein on AKT signaling should be considered hypothetical and requires further biochemical and cellular validation.

Aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that regulates a wide range of biological processes, including xenobiotic metabolism, immune responses, and cellular homeostasis. Upon ligand binding, AHR translocates to the nucleus and modulates the expression of target genes, such as those involved in detoxification pathways (Paris et al., 2021; Tsuji et al., 2014). Notably, AHR signaling is highly context-dependent and has been reported to exert both protective and adverse effects depending on the ligand type, cellular environment, and downstream signaling pathways (Wang et al., 2021). In this study, molecular docking (Fig. 7 and Table 4) suggested that 5-O-methylgenistein may bind within the

AHR binding pocket through interactions with residues GLN171, PHE170, LEU265, ALA133, TYR159, VAL129, and ALA264. However, this predicted binding does not indicate whether the compound acts as an agonist or antagonist, nor does it imply a specific biological outcome. Therefore, the potential role of 5-O-methylgenistein in modulating AHR signaling should be considered hypothetical and requires further functional validation.

Pharmacokinetics and toxicological prediction

The drug-likeness of the six flavonoid compounds from *P. macrocarpa* was initially evaluated using Lipinski's Rule of Five, followed by comprehensive ADMET profiling to assess their pharmacokinetic and safety properties. As shown in Table 2, all compounds satisfied the molecular weight (<500 Da) and lipophilicity (LogP <5) criteria, indicating general suitability for oral drug candidates. However, deviations were observed in hydrogen-bonding parameters, particularly for glycitin and catechin 7-O-β-D-xyloside, which exhibited higher counts of hydrogen-bond donors and acceptors due to glycosylation. This increased polarity is known to limit passive membrane permeability (Goetz et al., 2017).

These Lipinski-based predictions were strongly supported by the ADMET results (Table 3). Compounds that fully complied with Lipinski rules, such as 5-O-methylgenistein, naringenin, and 8-prenylnaringenin, demonstrated superior absorption profiles, including high Caco-2 permeability (log Papp > 1) and excellent human intestinal absorption (>90%). In contrast, catechin 7-O-β-D-xyloside, which exhibited multiple Lipinski violations, showed markedly lower intestinal absorption (43.20%), consistent with its higher polarity and reduced membrane permeability (Goetz et al., 2017). This highlights the impact of glycosylation on pharmacokinetics, particularly its role in limiting oral bioavailability.

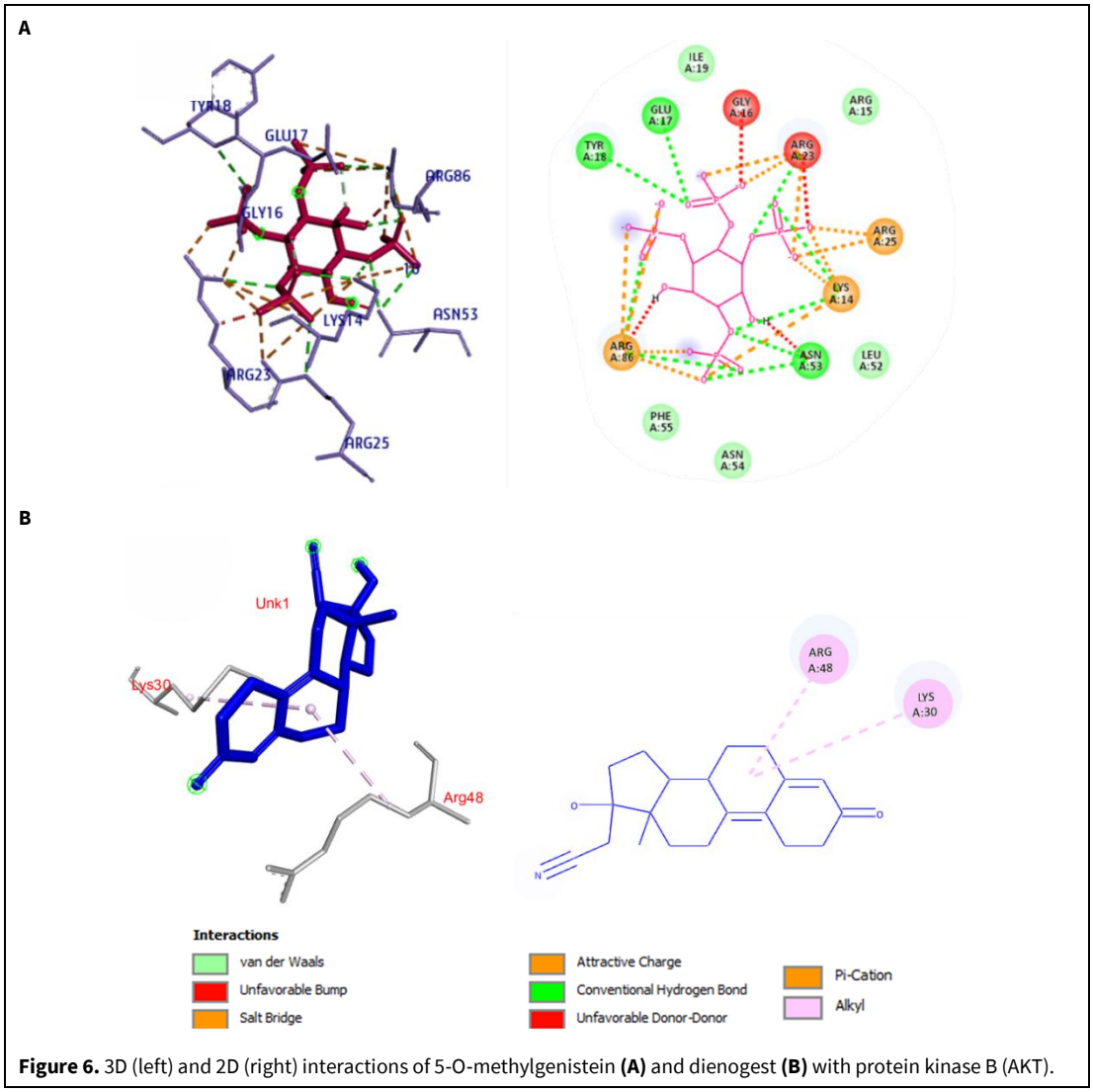


Figure 6. 3D (left) and 2D (right) interactions of 5-O-methylgenistein **(A)** and dienogest **(B)** with protein kinase B (AKT).

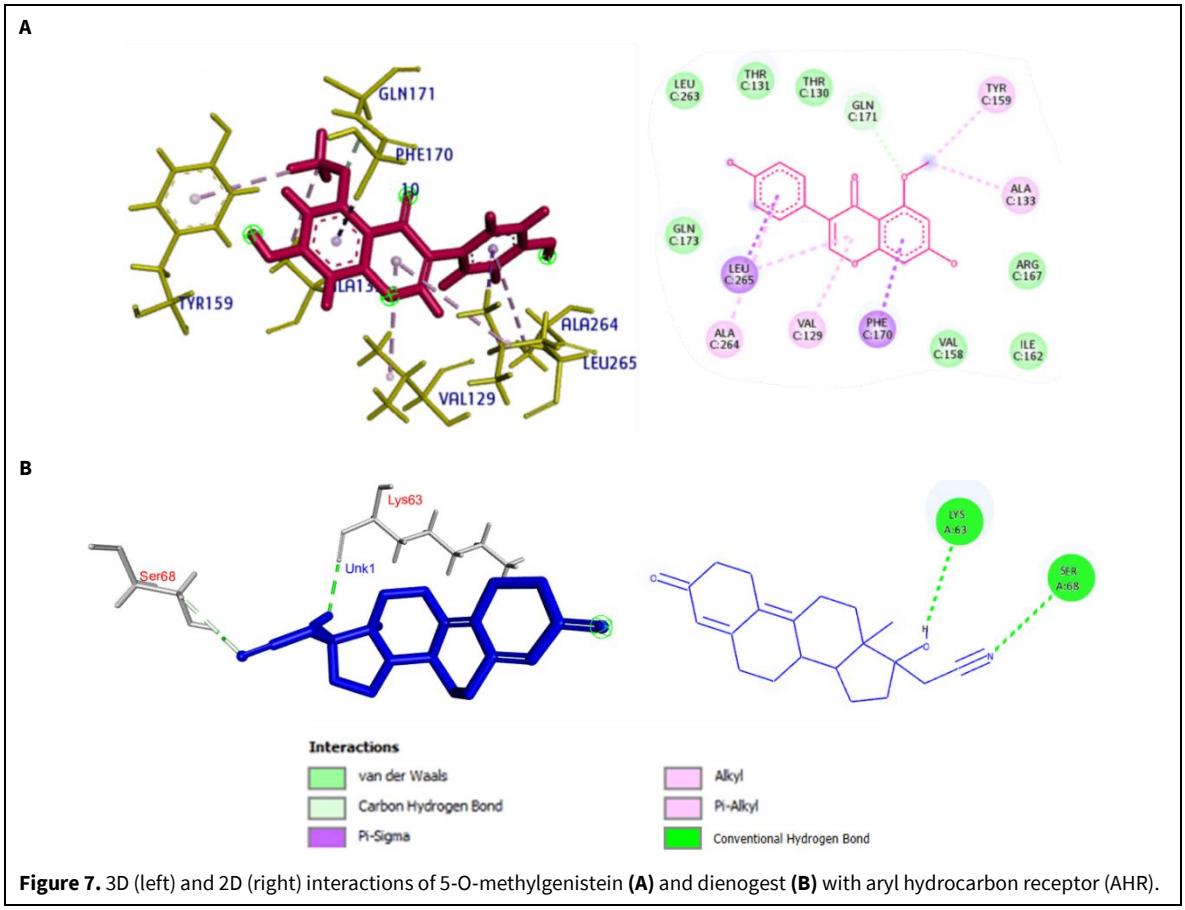


Figure 7. 3D (left) and 2D (right) interactions of 5-O-methylgenistein **(A)** and dienogest **(B)** with aryl hydrocarbon receptor (AHR).

Despite acceptable lipophilicity, all compounds were predicted to be substrates of P-glycoprotein (P-gp), suggesting that efflux mechanisms may reduce their effective absorption *in vivo*. Nevertheless, none of the compounds were predicted to be P-gp inhibitors, thereby reducing the likelihood of transporter-mediated drug–drug interactions (Ahmed Juvale et al., 2022). Distribution analysis further indicated limited central nervous system penetration for all compounds (log BB < −0.3), aligning with their physicochemical properties and suggesting a lower risk of CNS-related adverse effects (Pardridge, 2012).

Metabolic profiling revealed that 5-O-methylgenistein and 8-prenylnaringenin are substrates of CYP3A4, and that 5-O-methylgenistein is also predicted to inhibit multiple CYP isoforms (CYP1A2, CYP2C9, CYP2C19, and CYP3A4). While this broad interaction profile may raise concerns about drug–drug interactions, it also reflects the compound's metabolic reactivity (Hakkola et al., 2020), a characteristic commonly observed in flavonoid structures. In contrast, eriodictyol and catechin 7-O-β-D-xyloside showed minimal CYP inhibition, suggesting a more favorable metabolic safety profile.

From a toxicity perspective, all compounds were predicted to be non-mutagenic (AMES negative) and non-hepatotoxic, supporting their baseline safety as natural product–derived compounds. However, potential cardiotoxicity was identified, as glycitin, 5-O-methylgenistein, and 8-prenylnaringenin were predicted to be hERG II inhibitors. This finding indicates the need for further experimental validation to assess cardiac safety. Additionally, variations in maximum tolerated dose (MTD) and chronic toxicity (LOAEL) suggest differences in therapeutic windows among the compounds.

Overall, integration of Lipinski and ADMET analyses indicates that 5-O-methylgenistein possesses favorable absorption characteristics and acceptable drug-likeness, although its CYP inhibition profile and potential hERG liability warrant further investigation. Naringenin and eriodictyol exhibit more balanced pharmacokinetic and safety profiles, while catechin 7-O-β-D-xyloside, despite its safety advantages, may be limited by poor intestinal absorption. These findings emphasize that Lipinski compliance alone is insufficient to predict drug performance, and comprehensive ADMET evaluation is essential for prioritizing candidates for further development.

Importantly, these results should be interpreted as predictive and hypothesis-generating, and require experimental validation to confirm pharmacokinetic behavior and safety in biological systems.

Study limitation and future direction

This study presents a comprehensive *in silico* evaluation of flavonoid compounds from *P. macrocarpa* against endometriosis-relevant targets; however, several limitations should be acknowledged. The molecular docking approach employed in this study provides only a static representation of ligand–protein interactions and does not account for protein flexibility, solvent dynamics, or long-term binding stability. Although redocking validation was performed to ensure protocol reliability, the absence of molecular dynamics (MD) simulations limits the ability to confirm the stability and persistence of the predicted binding modes. Importantly, the study is limited by the absence of experimental validation. The biological activities proposed—including anti-inflammatory effects, modulation of estrogen receptor signaling, and involvement in AKT- and AHR-related pathways—are based on predictive modeling and should be interpreted as hypotheses rather than confirmed mechanisms. In particular, docking to caspase-3 cannot be directly translated into apoptosis induction without functional assays, and similar caution applies to all inferred mechanistic pathways.

Future studies should prioritize both computational and experimental validation to strengthen the translational relevance of these findings. From a computational perspective, the incorporation of MD simulations (e.g., 100–200 ns) for key complexes—particularly 5-O-methylgenistein with COX-2 and estrogen receptor—would allow assessment of binding stability through RMSD, RMSF, and hydrogen bond occupancy analyses. Free energy calculations using MM/PBSA or related methods are also recommended to provide a more rigorous energetic evaluation. In addition, consensus docking using multiple platforms and expanded selectivity analyses (e.g., COX-1 vs. COX-2, ERα vs. ERβ) would improve robustness.

From an experimental standpoint, *in vitro* validation represents a critical next step. Recommended assays include COX-2 enzymatic inhibition and PGE2 quantification; estrogen receptor binding or reporter assays; AKT phosphorylation analysis (e.g., Western blot for p-AKT); apoptosis assays (Annexin V/PI, caspase-3/7 activity); and measurement of inflammatory cytokines such as IL-6 and TNF-α in relevant endometriosis cell models. Furthermore, AHR functional assays (e.g., XRE-luciferase and CYP1A1 expression) would clarify the role of this pathway. *In vivo* validation using an endometriosis animal model should be conducted to evaluate lesion development, histopathology, angiogenesis (e.g., VEGF expression), pain-related behavior, and systemic safety.

CONCLUSION

5-O-Methylgenistein, one of the six flavonoid compounds evaluated, demonstrated favorable interaction profiles with multiple protein targets based on molecular docking analysis. These findings suggest that 5-O-methylgenistein may have potential anti-inflammatory and antineoplastic properties, as indicated by its predicted binding to key proteins involved in the pathophysiology of endometriosis. The docking results propose putative mechanisms whereby 5-O-methylgenistein may interact with COX-2, caspase-3, AKT, and AHR, which are associated with pathways related to pain modulation, apoptosis, cell proliferation, and early oncogenic processes. However, these mechanistic insights are based solely on computational predictions and should be interpreted as hypothesis-generating rather than definitive biological effects. Further *in vitro* and *in vivo* experimental studies are required to validate these predicted interactions and to confirm the therapeutic relevance of 5-O-methylgenistein in endometriosis.

CONFLICT OF INTEREST

The research was conducted entirely free from any involvement in commercial, financial, or personal matters that might be perceived as a possible conflict of interest or that could have influenced the outcomes or interpretation of the study.

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GENERATIVE ARTIFICIAL INTELLIGENCE (AI)

The authors used Grammarly to improve the language and readability of the manuscript. The authors reviewed and edited the content and take full responsibility for its accuracy. No images were generated or manipulated using AI tools.

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AUTHOR CONTRIBUTION:

Contribution	Sutrisno S	Maharani M
Concepts or ideas	x	x
Design	x	x
Definition of intellectual content	x	x
Literature search	x	
Experimental studies	x	x
Data acquisition	x	x
Data analysis	x	x
Statistical analysis	x	x
Manuscript preparation	x	x
Manuscript editing	x	x
Manuscript review	x	x

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